Possible Inhibitory Molecular Mechanism of Farnesol on the Development of Fluconazole Resistance in *Candida albicans* Biofilm

Li-hua Yu, Xin Wei, Ming Ma, Xiao-jun Chen, and Shuang-bo Xu

Institute of Stomatology, Department of Operative Dentistry and Endodontics, School of Stomatology, Nanjing Medical University, Nanjing, China

Candida albicans biofilm infections are usually treated with azole antifungals such as fluconazole. However, the development of resistance to this drug in *C. albicans* biofilms is very common, especially in immunocompromised individuals. The upregulation of the sterol biosynthetic pathway gene *ERG* and the efflux pump genes *CDR* and *MDR* may contribute to this azole tolerance in *Candida* species. We hypothesize that farnesol, an endogenous quorum sensing molecule with possible antimicrobial properties which is also the precursor of ergosterols in *C. albicans*, may interfere with the development of fluconazole resistance in *C. albicans* biofilms. To test this hypothesis, MICs were compared and morphology changes were observed by confocal laser scanning microscopy (CLSM) for farnesol-treated and -untreated and fluconazole-resistant groups. The expression of possible target genes (*ERG11*, *ERG25*, *ERG6*, *ERG5*, *ERG3*, *ERG1*, *MDR1*, *CDR1*, and *CDR2*) in biofilms was analyzed by reverse transcription-PCR (RT-PCR) and quantitative PCR (qPCR) to investigate the molecular mechanisms of the inhibitory effects of farnesol. The results showed a decreased MIC of fluconazole and thinner biofilms for the farnesol-treated group, indicating that farnesol inhibited the development of fluconazole resistance. The sterol biosynthetic pathway may contribute to the inhibitory effects of farnesol, as the transcription levels of the *ERG11*, *ERG25*, *ERG6*, *ERG3*, and *ERG1* genes decreased in the farnesol-treated group.

*C*andida albicans is an opportunistic fungus most commonly isolated from the oral cavity and the gastrointestinal and genitourinary tracts, as well as from the skin. *Candida* infection may happen in patients who have been immunocompromised or immune deficient, and the organism has various virulence traits that may cause diseases ranging from superficial mucosal infections to life-threatening systemic disorders. Furthermore, with the increasing use of antibiotics, hormones, and antitumor drugs, as well as biomaterials used in the mouth and body, such as stents, shunts, prostheses, implants, endotracheal tubes, pacemakers, and various types of catheter, the mortality and morbidity caused by *C. albicans* have risen year by year. Antifungal azoles such as fluconazole (oral and intravenous) and miconazole (topical) are used as treatment or prophylaxis for most *C. albicans* infections. However, treatment failures and infection recurrences are common due to increasing resistance to the antifungal azoles developed in *C. albicans* biofilms (2, 3, 12). It is crucial to explore novel compounds for therapeutic or preventive strategies targeting biofilm-related infections.

A biofilm is an organized community that is regulated by the exchange of chemical signals among cells in a process known as quorum sensing (QS). Quorum sensing refers to the molecular mechanism of regulation of gene expression in response to fluctuations in cell density (23). *C. albicans* produces and releases more quorum sensing molecules (QSM) in formed biofilms than during planktonic growth (1). Biofilm formation is more important than planktonic growth because this mode of growth is associated with the chronic nature of subsequent infections and with their inherent resistance to antifungal chemotherapy. A mature *C. albicans* biofilm with higher cell density displays more antifungal resistance than an early biofilm with lower cell density (27, 37). With the maturation of a biofilm and the increasing cell density, the production of QSM changes (1, 31, 42). These studies suggest that quorum sensing is one of the mechanisms for antifungal resistance in *C. albicans* biofilms.

Farnesol is an extracellular QSM produced by *C. albicans*; a certain concentration of farnesol inhibits the yeast-to-hypha transition and compromises biofilm formation (14). Farnesol keeps the *C. albicans* biofilm in stationary phase and inhibits its maturation (31). It is difficult for the organism to develop resistance to fluconazole before the maturation of a biofilm. In this study, we hypothesized that farnesol is a chemical compound that inhibits not only biofilm formation but also the development of fluconazole resistance.

In *C. albicans*, fluconazole resistance is a multifactorial process mediated through multiple underlying mechanisms (11, 35), including alterations in the target enzyme in the sterol biosynthetic pathway and increased efflux of the drug (6, 8, 10, 19, 20, 22, 26, 41). Studies have shown that farnesol is generated endogenously by enzymatic dephosphorylation of farnesyl diphosphate (FPP) (15, 16). FPP is a precursor for the synthesis of ergosterols and dolichols in the sterol biosynthesis pathway (9, 16, 21, 29, 38, 40). Antifungals (such as azoles) targeting the sterol biosynthetic pathway lead to increased levels of intracellular farnesol and also change the levels of extracellular farnesol (16). In addition, farnesol keeps *C. albicans* biofilms in stationary phase by inhibiting yeast growth and germ tube formation. As *C. albicans* approaches stationary phase, the expression of *ERG11* decreases (13). A correlation may exist between ergosterol biosynthesis and farnesol, in which farnesol may act as a chemical signaling molecule to regulate gene expression, resulting in inhibition of the development of fluconazole resistance in *C. albicans* biofilms.

In the present study, we studied the role of farnesol in the

inhibition of fluconazole resistance of *C. albicans* biofilms, as well as its molecular mechanisms. We measured the MIC to compare fluconazole resistances by using a formazan salt reduction assay with farnesol-treated and -untreated and fluconazole-resistant groups. The morphological changes of the biofilms in these 3 groups were also observed by confocal laser scanning microscopy (CLSM). The expression of possible target genes (*ERG11*, *ERG25*, *ERG6*, *ERG5*, *ERG3*, *ERG1*, *MDR1*, *CDR1*, and *CDR2*) was analyzed by using reverse transcription-PCR (RT-PCR) and quantitative PCR (qPCR) analyses to investigate the molecular mechanism for the inhibitory effects of farnesol on the development of fluconazole resistance.

MATERIALS AND METHODS

Organism and growth conditions. *C. albicans* strain SC5314 was kindly provided by the Department of Microbiology and Immunology, Second Military Medical University, Shanghai, China. Freshly grown yeast cells from Sabouraud's dextrose agar (SDA) plates were propagated in yeast-peptone-dextrose (YPD) medium and incubated overnight in an orbital shaker (75 rpm) at 30°C. The cells were collected by centrifugation ($2,100 \times g$, 10 min), washed in sterile phosphate-buffered saline (PBS; Sigma Chemical Co., St. Louis, MO), resuspended in RPMI 1640 supplemented with L-glutamine, and buffered with morpholinepropanesulfonic acid (Gibco Ltd., Paisley, United Kingdom). The solution was then adjusted to a cell density of 5×10^5 cells/ml for all experiments. All experiments were performed in triplicate on three separate occasions.

Biofilm formation and farnesol treatment. Biofilms of *C. albicans* were formed on a polystyrene surface following the protocol of Ramage et al. (32). One hundred microliters of standardized suspension was dispensed into flat-bottom 96-well microtiter plates (Corning Inc., NY) for drug susceptibility testing. In addition, 2 ml of suspension was inoculated into glass-bottom cell culture dishes (Corning Inc., NY) for CLSM observation. The plates and dishes were incubated at 37°C in a moist chamber. After 1 h of incubation, nonadherent cells were removed by thoroughly washing the biofilms three times with PBS.

Three groups (farnesol treated, farnesol untreated [control], and fluconazole resistant) were included in this study. The farnesol-treated sample was a biofilm formed by SC5314 and cultured in RPMI medium with 300 μM farnesol, the drug-resistant sample was a biofilm formed by a fluconazole-resistant isolate derived from strain SC5314 and cultured in RPMI medium without farnesol, and the farnesol-untreated control sample was a biofilm formed by SC5314 and cultured in RPMI medium without farnesol.

Stock solutions (100 mM) of farnesol (*E,E*-farnesol; Sigma Chemical Co., St. Louis, MO) were dissolved in 100% (vol/vol) methanol and frozen at −70°C until use (14, 24). Farnesol was diluted to a concentration of 300 μM in RPMI 1640 medium for experiments.

Induction of resistant strain. The activity of fluconazole against planktonic forms of *C. albicans* was evaluated by determining the MIC by using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution method (5). The revised breakpoints, which are much more sensitive for detecting emerging resistance (28), are as follows: susceptible (S), MIC of ≤2 μg/ml; susceptible but dose dependent (SDD), MIC of 4 μg/ml; and resistant (R), MIC of ≤8 μg/ml. In our study, the breakpoint for the resistant strain was chosen to be a MIC of ≤64 μg/ml (7a), which was better suited for the developed resistance study.

The protocol for induction of a resistant strain was the same as that previously described (33). A single randomly selected colony was inoculated into 5 ml RPMI 1640 medium containing 2× the MIC of fluconazole and incubated overnight with constant agitation at 30°C. An aliquot of 100 μl was then serially subcultured onto fresh SDA plates containing 4× the MIC of fluconazole for 48 h at 37°C. When the fluconazole MIC reached or exceeded 64 μg/ml, the cells were considered fluconazole resistant and were frozen in 30% glycerol (1:1 [vol/vol]) at −70°C for the

TABLE 1 Primers for RT-PCR analysis

Primer	Sequence (5′ → 3′)	Product size (bp)
ERG1-F	GCAACCGGCTGGTATCAAGGCA	273
ERG1-R	TGGTGAAATGCAGCCCCACGT	
ERG3-F	ACGTGCCACTACTGCCATTCCAG	453
ERG3-R	TGGACAGTGTGACAAGCGGTACC	
ERG5-F	GCCGTAGCCAAAGCAACTGGC	384
ERG5-R	ACGGCGGTAATCGGTGTGTTGT	
ERG6-F	AGATGTTGGTTGTGGTGTAGGTG	235
ERG6-R	AACTGGAGCATGAACGGTAGC	
ERG11-F	ACTACTCCAGTTTTCGGTAAAGGGGT	390
ERG11-R	CTTTTGAGCAGCATCACGTCTCCA	
ERG25-F	TGGATTGGCAGCAGAATATG	290
ERG25-R	TTTGGACCAGCTTCGGTATC	
CDR1-F	ACTCCTGCTACCGTGTTGTTATTG	192
CDR1-R	ACCTGGACCACTTGGAACATATTG	
CDR2-F	CTGTTACAACCACTATTGCTACTG	297
CDR2-R	TACCTTGGACAACTGTGCTTC	
MDR1-F	GGTGCTGCTACTACTGCTTCTG	226
MDR1-R	TGATGAAACCCAACACGGAACTAC	
18S rRNA-F	GGATTTACTGAAGACTAACTACTG	144
18S rRNA-R	GAACAACAACCGATCCCTAGT	

next experiment. When the fluconazole MIC was lower than 64 μg/ml, a single randomly selected colony was taken from the last SDA plate and inoculated into 5 ml RPMI 1640 medium containing a fluconazole concentration of twice the most recently measured MIC for that population. At each passage, aliquots were taken to determine the MIC until the fluconazole MIC reached or exceeded 64 μg/ml.

Susceptibility of biofilms to fluconazole. Three study groups were included in this project, including farnesol-treated and -untreated and fluconazole-resistant groups. The MIC was monitored for each study group before every experiment.

Biofilms were formed on the surfaces of 96-well, flat-bottom microtiter plates following the instructions described above. After 24 h at 37°C, fluconazole was added to the biofilms at serially 2-fold-diluted concentrations (1,024 to 1 μg/ml) and incubated for a further 24 h at 37°C. Fluconazole-free wells and biofilm-free wells were included to serve as controls. Sessile MICs (SMICs) were determined as previously described by using the XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide] reduction assay (KeyGEN Bio Co., Nanjing, China) (32). Colorimetric changes analyzed in a microtiter plate reader (BioTek Instruments, Inc., VT) at a wavelength of 450 nm indicated the changes of metabolic activity of the biofilm. The lowest drug concentrations that inhibited biofilm growth by 50% and 80% were considered the $SMIC_{50}$ and $SMIC_{80}$, respectively (39).

Comparison of biofilm formation by CLSM. Biofilms were formed on the glass bottom of cell culture dishes for CLSM observation. After 24 h of incubation at 37°C, the medium was aspirated, and the biofilms were washed three times with PBS. Fluconazole was then added to the biofilms at a concentration of 512 μg/ml, and the biofilms were incubated for a further 24 h at 37°C. Following incubation, the formed biofilms were washed with PBS and fixed with 4% paraformaldehyde. Biofilms were then stained with 500 μl calcofluor white stain (34) (0.0025 g/ml; Sigma Chemical Co., St. Louis, MO) for 30 min at 37°C in the dark and observed with a Zeiss LSM700 microscope with a video capture system, automatic camera, image analysis hardware and software (Carl Zeiss, Inc., Oberkochen, Germany), and a 405-nm argon ion laser.

Detection of possible target genes for development of resistance by RT-PCR and qPCR. Total RNA was isolated from 24-h *C. albicans* biofilms by a modified hot phenol method as previously described (4, 36). Reverse transcription was performed on 5 μg of total RNA by using avian

TABLE 2 Primers for qPCR analysis

Primer	Sequence (5′ → 3′)	Fragment size (bp)
ERG1-F	GCAACCGGCTGGTATCAAGGCA	183
ERG1-R	TCAACGGCATCAGGAACTGGCT	
ERG3-F	AAGATGGTGCTGTTCATG	157
ERG3-R	GGAATAGTTGCTGGGTTA	
ERG5-F	GCCGTAGCCAAAGCAACTGGC	117
ERG5-R	ACGGGGACCAGCAATTGAACCT	
ERG6-F	AGATGTTGGTTGTGGTGTAGGTG	235
ERG6-R	AACTGGAGCATGAACGGTAGC	
ERG11-F	AAGAATCCCTGAAACCAA	134
ERG11-R	CAGCAGCAGTATCCCATC	
ERG25-F	TGGATTGGCAGCAGAATATG	290
ERG25-R	TTTGGACCAGCTTCGGTATC	
CDR1-F	ACTCCTGCTACCGTGTTGTTATTG	192
CDR1-R	ACCTGGACCACTTGGAACATATTG	
CDR2-F	CTGTTACAACCACTATTGCTACTG	297
CDR2-R	TACCTTGGACAACTGTGCTTC	
MDR1-F	GGTGCTGCTACTACTGCTTCTG	226
MDR1-R	TGATGAAACCCAACACGGAACTAC	
18S rRNA-F	GGATTTACTGAAGACTAACTACTG	144
18S rRNA-R	GAACAACAACCGATCCCTAGT	

myeloblastosis virus (AMV) reverse transcriptase XL (Takara Bio Co., Ltd., Dalian, China) with random primers (Takara Bio Co., Ltd., Dalian, China) according to the manufacturer's instructions. Primers (Tables 1 and 2) were all designed by Shanghai Generay Bio-Tech Co., Ltd., taking 18S RNA as a reference. The synthetic cDNA was used for PCR on a PCR detection system (Eppendorf Bio, Hamburg, Germany). PCR conditions were as follows: denaturation (94°C, 2 min) followed by 30 cycles of denaturation (94°C, 30 s), annealing (55°C, 30 s), and extension (72°C, 45 s), ending with a 10-min extension at 72°C. PCR products were stained with 0.5 μg/ml ethidium bromide, separated by electrophoresis on 2.0% agarose gels in 1× TBE buffer (0.1 mol/liter Tris, 0.09 mol/liter boric acid, 1 mmol/liter EDTA, pH 8.4), and visualized under UV light.

The synthetic cDNA described above was also used for qPCR analysis, which was performed on an ABI 7500 Fast real-time PCR machine (Applied Biosystems, Rotkreuz, Switzerland), using Absolute QPCR SYBR green mix (Bio-Rad, Veenendaal, The Netherlands). Amplification was achieved using the following cycle settings: 2 min at 95°C followed by 40 cycles of 95°C for 10 s, 60°C for 30 s, and 72°C for 20 s. After amplification, a melting curve was analyzed to ensure the absence of primer dimers. Expression of genes was calculated using the $2^{-\Delta\Delta CT}$ method (16), using 18S RNA as a reference.

Statistical analysis. All experiments were performed in triplicate. One-way analysis of variance (ANOVA) was employed to assess the statistical significance of differences in matched samples. Differences were considered statistically significant for P values of <0.05. The analyses above were performed with SPSS Statistics 17.0 software (SPSS Inc., Chicago, IL).

The data were considered to represent differentially expressed genes in qPCR analysis if the values for $2^{-\Delta\Delta CT}$ were up- or downregulated at least

2.0-fold compared to the control group in three independent experiments.

RESULTS

Induction of fluconazole-resistant strain. When the MIC of fluconazole for *C. albicans* biofilms was >64 μg/ml, the strain was considered resistant to fluconazole. In this study, the MIC of our resistant strain of fluconazole was 128 μg/ml.

Different susceptibilities of biofilms to fluconazole. The SMIC$_{50}$ and SMIC$_{80}$ for each biofilm group are listed in Table 3. The SMIC$_{50}$ and SMIC$_{80}$ were lower for the farnesol-treated group than for the farnesol-untreated group and the fluconazole-resistant group, suggesting that farnesol inhibited *C. albicans* biofilm resistance to fluconazole.

Different morphological changes in biofilm formation. For the farnesol-untreated group, the biofilms formed consisted of extensively grown hyphae and pseudohyphae, and the thickness of the biofilms was about 228 μm (Fig. 1A). In contrast, the biofilms formed by the farnesol-treated group showed fewer hyphae but more pseudohyphae and spores. The thickness of the biofilms in this group was about 108 μm (Fig. 1B). Biofilms formed by the fluconazole-resistant group exhibited more hyphae and pseudohyphae, with many blastospores. The thickness was about 324 μm (Fig. 1C). These results indicated that the biofilms formed by the farnesol-treated group were thinner than those formed by the fluconazole-resistant and farnesol-untreated control groups.

Reduced expression of genes related to development of resistance. RT-PCR showed that the expression of *MDR1*, *ERG1*, *ERG3*, *ERG6*, *ERG11*, and *ERG25* was downregulated in the farnesol-treated group, the *ERG5* gene was significantly upregulated, and *CDR1/2* had no changes (Fig. 2). In contrast, the expression of *MDR1*, *ERG1*, *ERG3*, *ERG5*, *ERG11*, and *ERG25* was upregulated in the fluconazole-resistant group, while the expression of *CDR1/2* showed no difference from the control group (Fig. 2). qPCR further confirmed the results of RT-PCR, showing that the expression of *ERG1*, *ERG3*, *ERG6*, *ERG11*, *ERG25*, and *MDR1* in the farnesol-treated group was downregulated and the expression of *CDR1/2* was not different from that of the control group (Fig. 3 and 4). The expression of *ERG3*, *ERG5*, *ERG11*, *ERG25*, and *MDR1* was upregulated in the fluconazole-resistant group, while the expression of *CDR1* and *CDR2* in the fluconazole-resistant group was at similar levels to those for the control group (Fig. 3 and 4). These results suggest that farnesol downregulates *ERG* gene expression, resulting in a decrease of fluconazole resistance.

DISCUSSION

The formation of *C. albicans* biofilms enhances the ability of this fungus to develop resistance to fluconazole *in vitro* and in patients on fluconazole therapy. Resistance to fluconazole occurs by a combination of different molecular mechanisms, with the pre-

TABLE 3 SMICs of fluconazole for farnesol-treated biofilms, fluconazole-resistant biofilms, and farnesol-untreated biofilms at various times of development

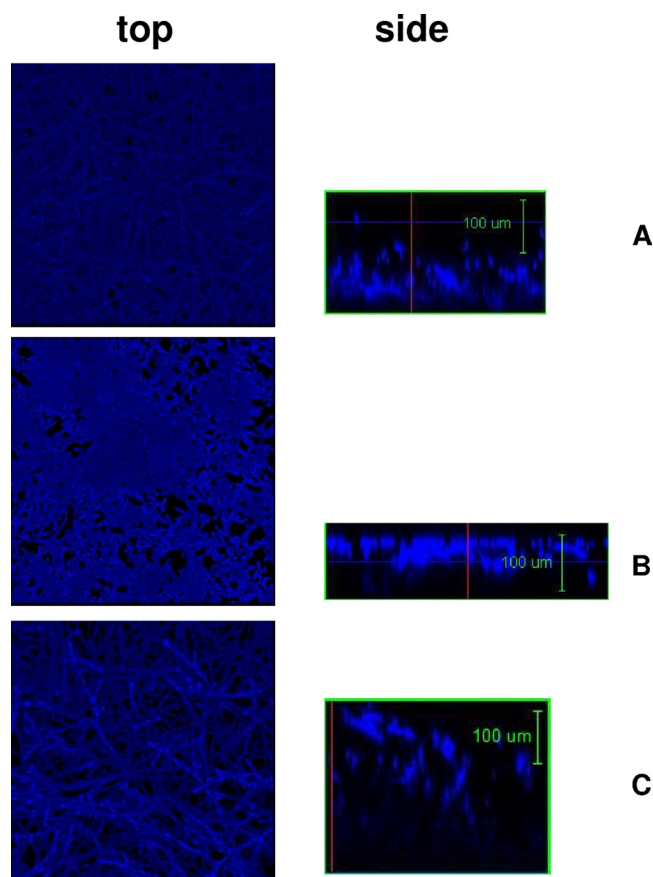| Group	6 h		12 h		18 h		24 h	
	SMIC$_{50}$	SMIC$_{80}$	SMIC$_{50}$	SMIC$_{80}$	SMIC$_{50}$	SMIC$_{80}$	SMIC$_{50}$	SMIC$_{80}$
Farnesol treatment group	1	16	1	64	2	128	4	256
Resistant group	512	>1,024	>1,024	>1,024	>1,024	>1,024	>1,024	>1,024
Control group	32	>1,024	256	>1,024	512	>1,024	>1,024	>1,024

top side

FIG 1 Three-dimensional reconstructions of *C. albicans* biofilms by CSLM and associated software for the compilation of *x-y* optical sections taken across the *z* axis. Magnification, ×200. Images show views from the top and side to depict biofilm thickness. (A) Farnesol-untreated biofilm exposed to fluconazole, consisting of extensively grown hyphae and pseudohyphae. (B) Farnesol-treated biofilm exposed to fluconazole, consisting of pseudohyphae and spores. (C) Resistant biofilm exposed to fluconazole, consisting of hyphae and pseudohyphae.

dominating one being the overexpression of efflux transporters alone or in combination with overexpression of the target enzyme, which alters the interaction between azole antifungal agents and the enzyme (6, 8, 10, 19, 20, 22, 26, 41). It is crucial to develop new
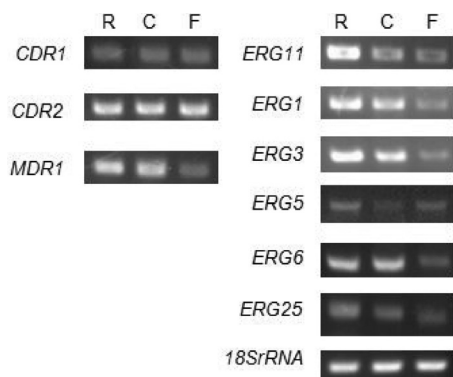

FIG 2 Electrophoretic images of PCR products, among which the bands for *CDR1/2* do not indicate differences among the three groups. R, resistant group; C, farnesol-untreated control group; F, farnesol-treated group.
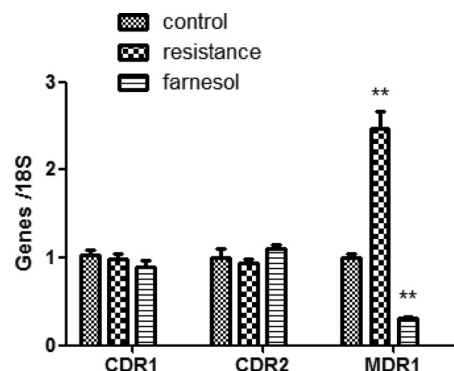

FIG 3 Expression of *CDR1/2* and *MDR1* determined by qPCR. Gene expression is indicated as the fold change relative to the control level. **, value for $2^{-\Delta\Delta CT}$ is >2 or <0.5, and *P* is <0.05 compared to the control group. *MDR1* was upregulated in the resistant group, while it was decreased in the farnesol-treated group.

strategies to overcome resistance, as well as new treatments for this condition.

Mature *C. albicans* biofilms are formed *in vitro* and *in vivo*, comprising a mixture of yeast cells, hyphae, and pseudohyphae in a dense network of organisms and water channels. Unlike the case for planktonic organisms, a mature biofilm is a community in which organisms are in contact with other organisms at a high density. *C. albicans* cells are more resistant to fluconazole in a mature biofilm than in planktonic form (30). A previous study showed that cell density has a role in *C. albicans* biofilm resistance (27, 37). Quorum sensing, which regulates cell density, is a possible mechanism of antifungal resistance of *C. albicans* biofilms. Farnesol is the first quorum sensing regulator found in eukaryotic cells (31). Since farnesol and its derivatives are precursors for the synthesis of ergosterols in the sterol synthetic pathway, exposure to exogenous farnesol may alter the balance of its intracellular levels. This will impact ergosterol biosynthesis and, in turn, the response of resistant strains to antifungals targeting ergosterols, such as fluconazole.

Studies have shown that farnesol affects the resistance of bacteria such as those in *Staphylococcus aureus* biofilms (17). A certain concentration of farnesol could inhibit the fluconazole resistance of resistant *C. dubliniensis* strains (18). The broth microdilution test demonstrated that the MIC values obtained for the *C. dublini-*
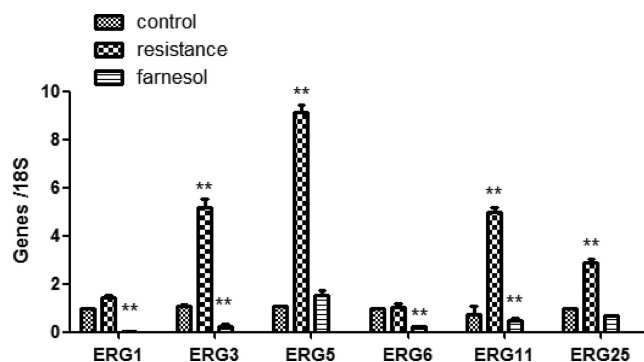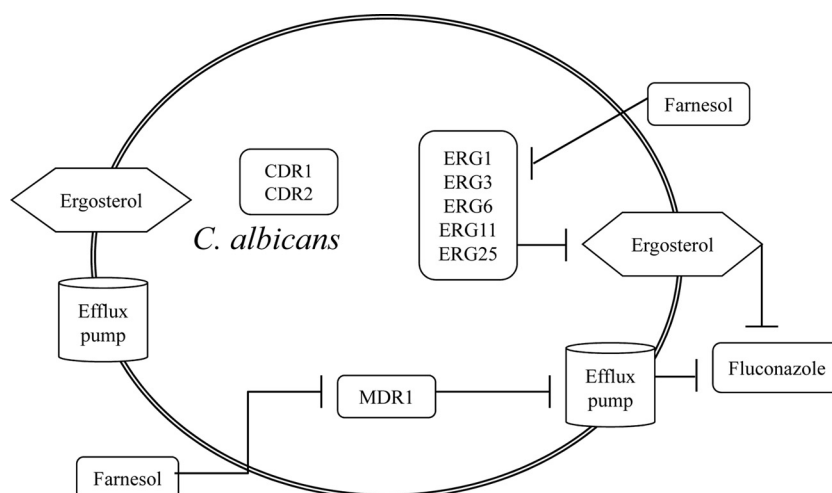

FIG 4 Expression of *ERG* genes determined by qPCR. Gene expression is indicated as the fold change relative to the control level. **, value for $2^{-\Delta\Delta CT}$ is >2 or <0.5, and *P* is <0.05 compared to the control group.

FIG 5 Diagrammatic sketch of our hypothesis. Farnesol interferes with the development of fluconazole resistance in *C. albicans* by regulating target gene expression.

ensis strains tested were inversely proportional to farnesol concentrations (18). In this study, we investigated the inhibitory role of farnesol in the fluconazole resistance of *C. albicans* biofilms and the mechanism of this inhibition. Results from an XTT assay suggested that the $SMIC_{50}$ and $SMIC_{80}$ of fluconazole for farnesol-treated biofilm were lower than those for the control and resistant biofilms. This confirms that farnesol inhibits the fluconazole resistance of *C. albicans*, which agrees with the previous study (18). CLSM images show that after exposure to fluconazole, the farnesol-treated biofilm was thinner than the control and resistant biofilms. Since farnesol inhibits the yeast-hypha transformation and biofilm formation (31), it leads to the formation of a thinner biofilm that is more easily compromised by fluconazole than a thicker one.

Previous studies showed that farnesol is the intermediate product of cytomembrane ergosterol synthesis, and its level is inversely proportional to the ergosterol synthesis level (15). Many antiseptics focus on ergosterol synthesis and inhibit fungi by changing the expression of genes and proteins, including those in the sterol synthesis pathway (7). *ERG* genes regulate ergosterol synthesis by modulating target enzymes in the sterol synthesis pathway. Reports have shown that fluconazole itself contributes to upregulation of *ERG1*, *ERG3*, *ERG11*, and *ERG25*, which was supposed to induce phenotypic resistance (25). In this study, the results of RT-PCR and qPCR showed that the levels of *ERG11*, *ERG1*, *ERG3*, *ERG6*, and *ERG25* were downregulated in farnesol-treated biofilms compared to the control and resistant biofilms. *ERG3*, *ERG5*, *ERG11*, and *ERG25* were obviously upregulated in the resistant biofilms. These results suggest that farnesol inhibits the drug resistance of *C. albicans* biofilms by effecting partial gene expression in ergosterol biosynthesis (Fig. 5).

It was found that there was no significant difference in expression of *CDR1/2* among the farnesol-treated group, the fluconazole-resistant group, and the untreated control group. However, the expression of *MDR1* was downregulated in the farnesol-treated group compared to the resistant group and the control group, suggesting that farnesol inhibits the drug resistance of *C. albicans* biofilms by playing a certain role in regulating the expression of multidrug resistance genes.

In conclusion, farnesol, a QSM secreted by *C. albicans* itself, affects the formation of biofilms and inhibits biofilm resistance. Inhibitory regulation was found not only on *ERG* genes but also on the *MDR1* gene, an important multidrug resistance gene in *C. albicans*. Farnesol might be used as a new drug to reduce *C. albicans* biofilm resistance and enhance the effects of fluconazole treatment in the future.

ACKNOWLEDGMENTS

REFERENCES

1. **Alem MAS, Oteef MDY, Flowers TH, Douglas LJ.** 2006. Production of tyrosol by *Candida albicans* biofilms and its role in quorum sensing and biofilm development. Eukaryot. Cell **5**:1770–1779.
2. **Al-Fattani MA, Douglas LJ.** 2004. Penetration of *Candida* biofilms by antifungal agents. Antimicrob. Agents Chemother. **48**:3291–3297.
3. **Al-Fattani MA, Douglas LJ.** 2006. Biofilm matrix of *Candida albicans* and *Candida tropicalis*: chemical composition and role in drug resistance. J. Med. Microbiol. **55**:999–1008.
4. **Andes D, et al.** 2004. Development and characterization of an in vivo central venous catheter *Candida albicans* biofilm model. **72**:6023–6031.
5. **Arikan S.** 2007. Current status of antifungal susceptibility testing methods. Med. Mycol. **45**:569–587.
6. **Asai K, et al.** 1999. Formation of azole-resistant *Candida albicans* by mutation of sterol 14-demethylase P450. Antimicrob. Agents Chemother. **43**:1163–1169.
7. **Borecká-Melkusová S, et al.** 2009. The expression of genes involved in the ergosterol biosynthesis pathway in *Candida albicans* and *Candida dubliniensis* biofilms exposed to fluconazole. Mycoses **52**:118–128.
8. **De Backer MD, et al.** 2001. Genomic profiling of the response of *Candida albicans* to itraconazole treatment using a DNA microarray. Antimicrob. Agents Chemother. **45**:1660–1670.
9. **Edwards PA, Ericsson J.** 1999. Sterols and isoprenoids: signaling molecules derived from the cholesterol biosynthetic pathway. Annu. Rev. Biochem. **68**:157–185.
10. **Favre B, Didmon M, Ryder NS.** 1999. Multiple amino acid substitutions in lanosterol 14α-demethylase contribute to azole resistance in *Candida albicans*. Microbiology **145**:2715–2725.
11. **Franz R, Ruhnke M, Morschhäuser J.** 1999. Molecular aspects of fluconazole resistance development in *Candida albicans*. Mycoses **42**:453–458.

12. **Hasan F, Xess I, Wang X, Jain N, Fries BC.** 2009. Biofilm formation in clinical *Candida* isolates and its association with virulence. Microbes Infect. **11**:753–761.

13. **Henry KW, Nickels JT, Edlind TD.** 2000. Up-regulation of ERG genes in *Candida* species by azoles and other sterol biosynthesis inhibitors. Antimicrob. Agents Chemother. **44**:2693–2700.

14. **Hornby JM, et al.** 2001. Quorum sensing in the dimorphic fungus *Candida albicans* is mediated by farnesol. Appl. Environ. Microbiol. **67**:2982–2992.

15. **Hornby JM, Kebaara BW, Nickerson KW.** 2003. Farnesol biosynthesis in *Candida albicans*: cellular response to sterol inhibition by zaragozic acid B. Antimicrob. Agents Chemother. **47**:2366–2369.

16. **Hornby JM, Nickerson KW.** 2004. Enhanced production of farnesol by *Candida albicans* treated with four azoles. Antimicrob. Agents Chemother. **48**:2305–2307.

17. **Jabra-Rizk MA, Meiller TF, James CE, Shirtliff ME.** 2006. Effect of farnesol on *Staphylococcus aureus* biofilm formation and antimicrobial susceptibility. Antimicrob. Agents Chemother. **50**:1463–1469.

18. **Jabra-Rizk MA, Shirtliff M, James C, Meiller T.** 2006. Effect of farnesol on *Candida dubliniensis* biofilm formation and fluconazole resistance. FEMS Yeast Res. **6**:1063–1073.

19. **Liu TT, et al.** 2005. Genome-wide expression profiling of the response to azole, polyene, echinocandin, and pyrimidine antifungal agents in *Candida albicans*. Antimicrob. Agents Chemother. **49**:2226–2236.

20. **Lupetti A, Danesi R, Campa M, Del Tacca M, Kelly S.** 2002. Molecular basis of resistance to azole antifungals. Trends Mol. Med. **8**:76–81.

21. **Machida K, Tanaka T, Fujita K, Taniguchi M.** 1998. Farnesol-induced generation of reactive oxygen species via indirect inhibition of the mitochondrial electron transport chain in the yeast *Saccharomyces cerevisiae*. J. Bacteriol. **180**:4460–4465.

22. **Manavathu EK, Kallakuri S, Arganoza MT, Vazquez JA.** 1999. Amino acid variations of cytochrome P-450 lanosterol 14α-demethylase (CYP51A1) from fluconazole-resistant clinical isolates of *Candida albicans*. Rev. Iberoam. Micol. **16**:198–203.

23. **March JC, Bentley WE.** 2004. Quorum sensing and bacterial cross-talk in biotechnology. Curr. Opin. Biotechnol. **15**:495–502.

24. **Melanie LL, Hasim S, Nickerson KW, Atkin AL.** 2010. Activity and toxicity of farnesol towards *Candida albicans* are dependent on growth conditions. Antimicrob. Agents Chemother. **54**:940–942.

25. **Nailis H, Vandenbosch D, Deforce D, Nelis HJ, Coenye T.** 2010. Transcriptional response to fluconazole and amphotericin B in *Candida albicans* biofilms. Res. Microbiol. **161**:284–292.

26. **Niimi M, Firth NM, Cannon RD.** 2010. Antifungal drug resistance of oral fungi. Odontology **98**:15–25.

27. **Perumal P, Mekala S, Chaffin WL.** 2007. Role for cell density in antifungal drug resistance in *Candida albicans* biofilms. Antimicrob. Agents Chemother. **51**:2454–2463.

28. **Pfaller MA, Andes D, Diekema DJ, Espinel-Ingroff A, Sheehan D.** 2010. Wild-type MIC distributions, epidemiological cutoff values and species-specific clinical breakpoints for fluconazole and *Candida*: time for harmonization of CLSI and EUCAST broth microdilution methods. Drug Resist. Updat. **13**:180–195.

29. **Plochocka D, Karst F, Swiezewska E, Szkopinska A.** 2000. The role of ERG20 gene (encoding yeast farnesyl diphosphate synthase) mutation in long dolichol formation. Molecular modeling of FPP synthase. Biochimie **82**:733–738.

30. **Potera C.** 1999. Forging a link between biofilms and disease. Science **283**:1837–1839.

31. **Ramage G, Saville SP, Wickes BL, Lopez-Ribot JL.** 2002. Inhibition of *Candida albicans* biofilm formation by farnesol, a quorum-sensing molecule. Appl. Environ. Microbiol. **68**:5459–5463.

32. **Ramage G, Vande WK, Wickes BL, Lopez-Ribot JL.** 2001. Standardized method for in vitro antifungal susceptibility testing of *Candida albicans* biofilms. Antimicrob. Agents Chemother. **45**:2475–2479.

33. **Ribeiro MA, Paula CR.** 2007. Up-regulation of ERG11 gene among fluconazole-resistant *Candida albicans* generated in vitro: is there any clinical implication. Diagn. Microbiol. Infect. Dis. **57**:71–75.

34. **Richard ML, Nobile CJ, Bruno VM, Mitchell AP.** 2005. *Candida albicans* biofilm-defective mutants. Eukaryot. Cell **4**:1493–1502.

35. **Sanglard D, Odds FC.** 2002. Resistance of *Candida* species to antifungal agents: molecular mechanisms and clinical consequences. Lancet Infect. Dis. **2**:73–85.

36. **Schmitt ME, Brown TA, Trumpower BL.** 1990. A rapid and simple method for preparation of RNA from *Saccharomyces cerevisiae*. Nucleic Acids Res. **18**:3091–3092.

37. **Seneviratne CJ, Jin LJ, Samaranayake YH, Samaranayake LP.** 2008. Cell density and cell aging as factors modulating antifungal resistance of *Candida albicans* biofilms. Antimicrob. Agents Chemother. **52**:3259–3266.

38. **Song L.** 2003. Detection of farnesyl diphosphate accumulation in yeast ERG9 mutants. Anal. Biochem. **317**:180–185.

39. **Shuford JA, Piper KE, Steckelberg JM, Patel R.** 2007. In vitro biofilm characterization and activity of antifungal agents alone and in combination against sessile and planktonic clinical *Candida albicans* isolates. Diagn. Microbiol. Infect. Dis. **57**:277–281.

40. **Szkopinska A, et al.** 1997. Polyprenol formation in the yeast *Saccharomyces cerevisiae*: effect of farnesyl diphosphate synthase overexpression. J. Lipid Res. **38**:962–968.

41. **Walsh TJ, et al.** 2000. Correlation between in vitro and in vivo antifungal activities in experimental fluconazole-resistant oropharyngeal and esophageal candidiasis. Antimicrob. Agents Chemother. **38**:2369–2373.

42. **Wei X, et al.** 2010. The role of cell density in the morphology of the *Candida albicans* biofilms development and the tyrosol production. Chinese J. Microbiol. Immunol. **30**:344–348.